IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of

Mamoru OHASHI et al — Group Art Unit: 1616

Serial No.: 09/529,715 — Examiner: Sharmila S. Gollamudi

Filed: April 19, 2000

For: FAST-DISSOLVING PHARMACEUTICAL COMPOSITION

DECLARATION

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

I, Mitsugu SANJOBA, a citizen of Japan residing at 3-12-18, Shimizugaoka, Sumiyoshi-ku, Osaka, Japan, declare as follows.

1. I was graduated from Kanazawa University, Faculty of Pharmaceutical Sciences in March 1971, and was qualified as a pharmaceutical chemist in May 1972.

Since April 1971 I have been an employee of Dainippon Pharmaceutical Co., Ltd. and had been engaged in research works with respect to analytical chemical research and physicochemical research, and from June, 1990 till May, 1998, I was assigned as a Chief researcher of Department of Physico Chemical Analysis, Exploratory Research Laboratories of said company. Since June 1998 up till the present I have been temporarily transferred to Nichiei Sangyo Co., Ltd., which is a solely owned subsidiary of Dainippon Pharmaceutical Co., Ltd. and assigned therein as a Director of Analytical Chemistry Center of said

company.

2. I have read the description of U.S. Application Serial No. 09/529,715 and am familiar with the subject matter thereof.

3. I have read the cited Negoro et al., U.S. Patent 5,258,382 and am familiar with the subject matter thereof.

4. When I was a Chief researcher in Exploratory Research Laboratories, Department of Physico Chemical Analysis of Dainippon Pharmaceutical Co., Ltd. in 1994, under my supervision, the following tests of physicochemical properties of AS-3201 had been done, and thereafter, I and co-authors had submitted to Dainippon Pharmaceutical Co., Ltd. a report entitled "Quality Test and Stability of SX-3201" on November 16, 1994. The following two tests: (1) Solubility Test and (2) Test of Partition Coefficient are a part of the report, wherein the test compound code number "SX-3201" is an identical compound to "AS-3201" indicated in Example 22, Negoro et al., U.S. Patent 5,258,382.

(1) Solubility Test:

Method:

According to the method described in The Japanese Pharmacopoeia, Twelfth Edition, General Notices 24, the solubility of SX-3201 in various solvents was tested. The method is outlined below.

Using SX-3201 (AS-3201) (Lot No. R93003), the test was carried out as follows.

To a certain amount of SX-3201 was added various solvents as shown in the following table and the amount of the solvent required for completely dissolving SX-3201 was determined, wherein the mixture of SX-3201 and the solvent was strongly shaken at 25°C or at room temperature for 30 seconds at an interval of 5 minutes within 30 minutes.

Results:

The results are shown in the following Table I, wherein the

solubility (mg/mL) is calculated as an amount (mg) of SX-3201 dissolved in the solvent (1 mL).

Table I

Solvent	Solubility (mg/mL)	Temperature
Water	<0.05 (final pH 5.9)	25°C
DMSO*	>100	25°C
Acetone	>100	25°C
Acetonitrile	>100	Room Temperature
Methanol	31	Room Temperature
Glacial acetic acid	26	Room Temperature
Ethanol (99.5%)	12	Room Temperature
Chloroform	7.8	Room Temperature
n-Octanol	2.0	Room Temperature

*) DMSO: Dimethyl sulfoxide

Based on the above test results, "amount of the solvent required for dissolving 1 g of solute (SX-3201)" was calculated and the data are shown in the following Table II. Besides, the solubility was evaluated along with "Descriptive term" for expressing the solubility as defined in General Notices 24 of The Japanese Pharmacopoeia, Twelfth Edition. The evaluation of solubility is also shown in Table II.

Table II

Solvent	Solvent required for dissolving 1 g of Solute	Descriptive term of solubility
Water	20,000 mL<	Practically insoluble
DMSO*	10 mL>	Very soluble or freely soluble
Acetone	10 mL>	Very soluble or freely soluble
Acetonitrile	10 mL>	Very soluble or freely soluble
Methanol	32 mL	Sparingly soluble
Glacial acetic acid	38 mL	Sparingly soluble
Ethanol (99.5%)	83 mL	Sparingly soluble
Chloroform	128 mL	Slightly soluble
n-Octanol	500 mL	Slightly soluble

*) DMSO: Dimethyl sulfoxide

As is clear from the above test results, SX-3201 is very soluble in DMSO, acetone and acetonitrile, but as to solubility in water, the amount

of the water required for dissolving 1 g of SX-3201 is more than 20,000 mL and it is valuate that the solubility of SX-3201 in water is "practically insoluble" in the descriptive term as defined in The Japanese Pharmacopoeia.

(2) Test of Partition Coefficient:

Method:

Using SX-3201 (AS-3201) (Lot No. R93004), the test was carried out as follows.

To a mixture of SX-3201 (about 30 μg/mL) and n-octanol (10 mL) was added 0.1 M phosphate buffer (pH 7.2, 10 mL), and the mixture was shaken at 37°C for one hour, and then subjected to centrifugation at 2000 r.p.m. for 10 minutes to separate the n-octanol layer and the buffer layer. With respect to both of the n-octanol layer and the buffer layer, the absorbance was measured at a maximum wave (λmax) of about 300 nm (the λmax of SX-3201 in n-octanol layer was 294 nm, and the λmax of SX-3201 in buffer layer was 302 nm). Based on the data thus obtained, the partition coefficient was calculated by the ratio of SX-3201 contained in the n-octanol layer and the buffer layer.

Results:

The results of the above test are shown in the following Table III.

Table III

	Absorbance at λmax	Partition Coefficient (P) at 37°C (n-Octanol/Buffer, pH 7.2)	Log P
n-Octanol layer	0.744	74	1.9
Phosphate buffer layer	0.01		

As is shown in the above, in the mixture of n-octanol and a 0.1M phosphate buffer (pH 7.2), SX-3201 was almost contained in the n-octanol layer and only slight amount of SX-3201 was moved into the buffer layer. Thus, the ratio of SX-3201 in both layers was 74 : 1, and the partition coefficient was 74.

5. It is my opinion, based upon my knowledge and experience in this field, that as shown in the above tests, SX-3201 (AS-3201 mentioned in Negoro et al., U.S. Patent 5,258,382) is very soluble in

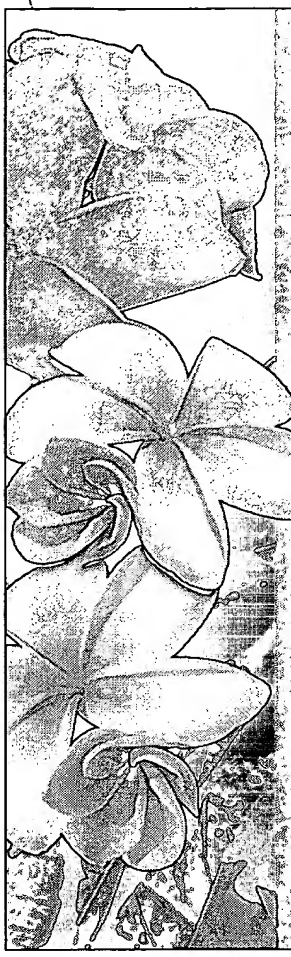
dimethyl sulfoxide, acetone and acetonitrile but is practically insoluble in water, and it has a partition coefficient of 74 in n-octanol and the phosphate buffer, and in view of these properties of SX-3201 (AS-3201), it may be concluded that said compound is classified into a hydrophobic compound.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 11 day of August, 2004.

Mitsugu Sanjoba

Mitsugu SANJOBA



Polyol Pathway Inhibition by AS-3201 in Sural Nerve from Patients with Diabetic Sensorimotor Polyneuropathy

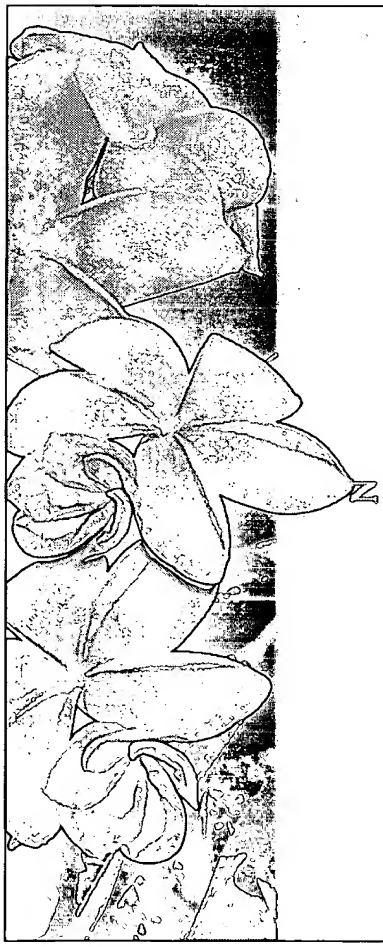
Dr. Vera Bril
The University Health Network
University of Toronto

AS-3201 Inhibition of the Polyol Pathway in Human Sural Nerve

- 12 week, randomized, double-blind
- Placebo, 5 mg/d, 20 mg/d AS-3201
- Sural nerve biopsy at 12 weeks
  Sural nerve sorbitol, fructose, AS-3201
- RBC sorbitol, fructose
- Plasma AS-3201 levels
- NCS, TCNS, VPT

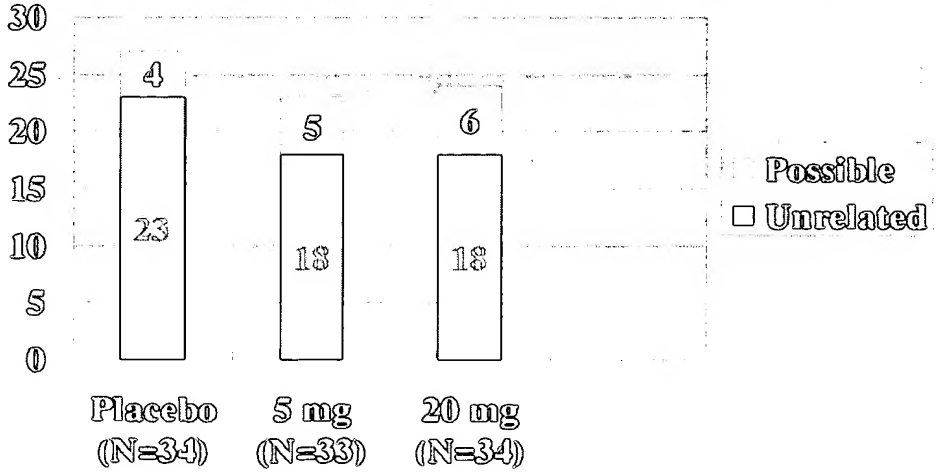
Patient Flow

- Screened 295
- Randomized 101
  - Placebo 34
  - AS-3201 5 mg/d 33
  - AS-3201 20 mg/d 34
- Drop-outs (including withdrawals due to protocol violation):
  - 2 Placebo
  - 2 AS-3201 5 mg/d
  - 6 AS-3201 20 mg/d

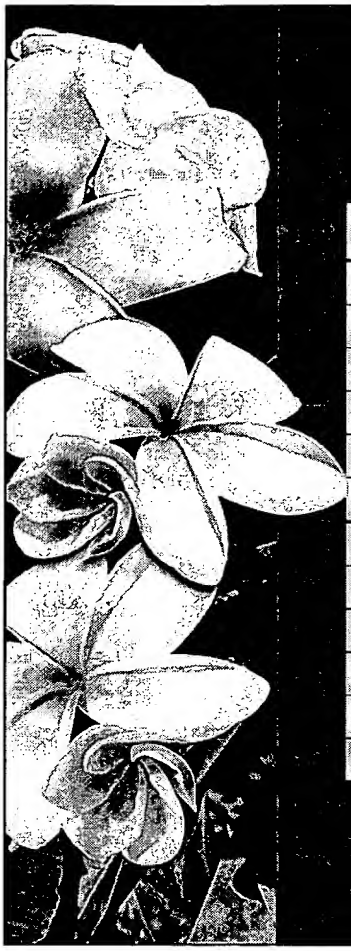


All Adverse Events

Number of Treatment-Emergent Adverse Events



No Probable or definite relationship to the study drug was reported in any groups.



Patient Demographic Profile

	Placebo	AS-3201 5 mg	AS-3201 20 mg	P-value
Number	34	33	34	
Gender N (%)				
Male	20 (58.8)	24 (72.7)	22 (64.7)	0.487
Female	14 (41.2)	9 (27.3)	12 (35.3)	
Age (y) *	58.1 ± 8.7	60.8 ± 7.6	56.9 ± 9.3	0.222
Race N (%)				
Caucasian	23 (67.6)	27 (81.8)	23 (67.6)	0.475
Hispanic	2 (5.9)	3 (9.1)	4 (11.8)	
African American	3 (8.8)	2 (6.1)	5 (14.7)	
Asian	3 (8.8)	1 (3.0)	1 (2.9)	
Other	3 (8.8)		1 (2.9)	
Weight, kg*	91.4 ±15.5	92.7 ± 16.4	92.2 ± 21.0	0.866

[1] P-values are based on the X^2 test for gender, race and type of diabetes, and the Kruskal-Wallis test for mean age, weight and other parameters.

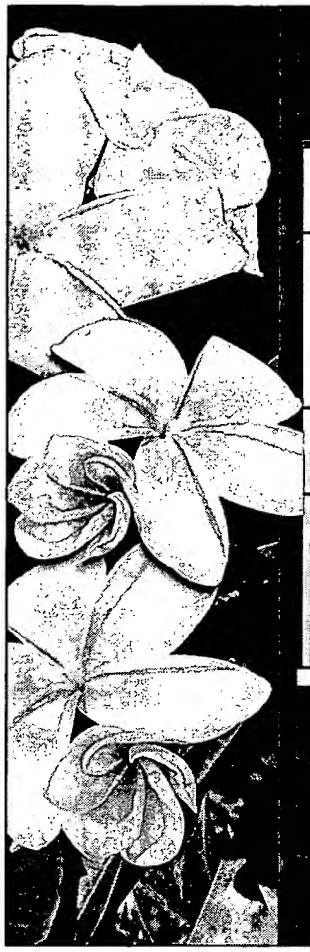
*values presented as mean ± standard deviation

Patient Demographic Profile

	Placebo	AS-3201 5 mg	AS-3201 20 mg	P-value
Type of Diabetes N (%)				
Type I	2 (5.9)	3 (9.1)	5 (14.7)	
Type II	32 (94.1)	30 (90.9)	29 (85.3)	0.468
Duration of Diabetes (y)*	15.4 ± 10.1	14.2 ± 8.8	14.7 ± 11.8	0.764
Duration of DSP (y)*	4.4 ± 3.2	3.9 ± 3.3	3.9 ± 4.4	0.289
Fasting Glucose (mg/dL)*	182.3 ± 69.2	169.1 ± 43.8	151.2 ± 62.2	0.455
Baseline HbA_1c (%)*	8.49 ± 1.52	8.18 ± 1.18	8.43 ± 1.27	0.720

[1] P-values are based on the χ^2 test for gender, race and type of diabetes, and the Kruskal-Wallis test for mean age, weight and other parameters.

*values presented as mean ± standard deviation



Clinical Neuropathy Score

Clinical Criteria		Maximum
Symptoms	Foot pain Numbness Tingling Weakness Imbalance Upper Limb symptoms	6 points
Reflexes	Ankle Reflex Knee Reflex	8 points
Signs	Pinprick Temperature Light touch Vibration Position sense	5 points

0-5 No neuropathy
6-8 Mild
9-11 Moderate
·12 Severe

Baseline NCS – Sensory Results

	Placebo	Treatment Group AS-3201 5 mg	AS-3201 20 mg	P-value
Sural, Right				
Amplitude	5.0 ± 4.4	3.9 ± 2.4	4.9 ± 4.0	0.708
Velocity	42.5 ± 4.5	41.7 ± 5.1	40.2 ± 5.3	0.087
Sural, Left				
Amplitude	5.1 ± 4.4	3.9 ± 2.5	4.4 ± 2.8	0.831
Velocity	42.2 ± 4.1	41.7 ± 5.7	39.9 ± 4.5	0.083
Median Sensory, Proximal				
Amplitude	7.8 ± 4.6	7.3 ± 3.9	8.0 ± 5.1	0.806
Velocity	54.9 ± 5.1	55.4 ± 4.8	53.9 ± 4.6	0.203
Median Sensory, Distal				
Amplitude	16.7 ± 11.3	16.3 ± 8.8	15.6 ± 9.9	0.922
Velocity	46.3 ± 10.5	49.1 ± 7.1	48.2 ± 6.0	0.662

[1]P-values are based on the Kruskal-Wallis test. * $p < 0.05$

All conduction velocities are in meters/second; sensory potential amplitudes in μV.

Baseline NCS – Motor Results

	Placebo	Treatment Group AS-3201 5 mg	AS-3201 20 mg	P-value
Median Motor				
Amplitude, Wrist	8.9 ± 3.0	9.0 ± 2.6	9.6 ± 3.2	0.538
Velocity	50.0 ± 4.0	52.4 ± 3.4	49.7 ± 4.1	0.007*
Peroneal Motor				
Amplitude, Knee	3.8 ± 2.1	3.6 ± 1.7	4.1 ± 2.3	0.582
Velocity	39.8 ± 3.0	40.7 ± 3.3	38.9 ± 4.7	0.330
F-wave Latency				
Median	30.4 ± 3.0	29.3 ± 2.7	30.2 ± 3.2	0.387
Peroneal	55.3 ± 6.6	54.6 ± 6.9	55.7 ± 7.3	0.769

[1]P-values are based on the Kruskal-Wallis test. * $p < 0.05$

All conduction velocities are in meters/second; sensory potential amplitudes in μV, and motor potential amplitudes in mV, F wave latencies are in msec.

Baseline VPT & Toronto Clinical Neuropathy Score Results

	Placebo	Treatment Group AS-3201 5 mg	AS-3201 20 mg	P-value
Vibration Perception Threshold				
Right Toe	20.3 ± 8.0	22.4 ± 9.3	21.2 ± 6.6	0.679
Left Toe	20.9 ± 8.8	21.7 ± 9.6	20.5 ± 6.5	0.962
Toronto Clinical Neuropathy Score	10.4 ± 3.5	10.2 ± 3.0	9.9 ± 4.2	0.729

[1]P-values are based on the Kruskal-Wallis test. * $p < 0.05$

. The vibration perception thresholds are in volts.

Plasma Concentrations of AS-3201

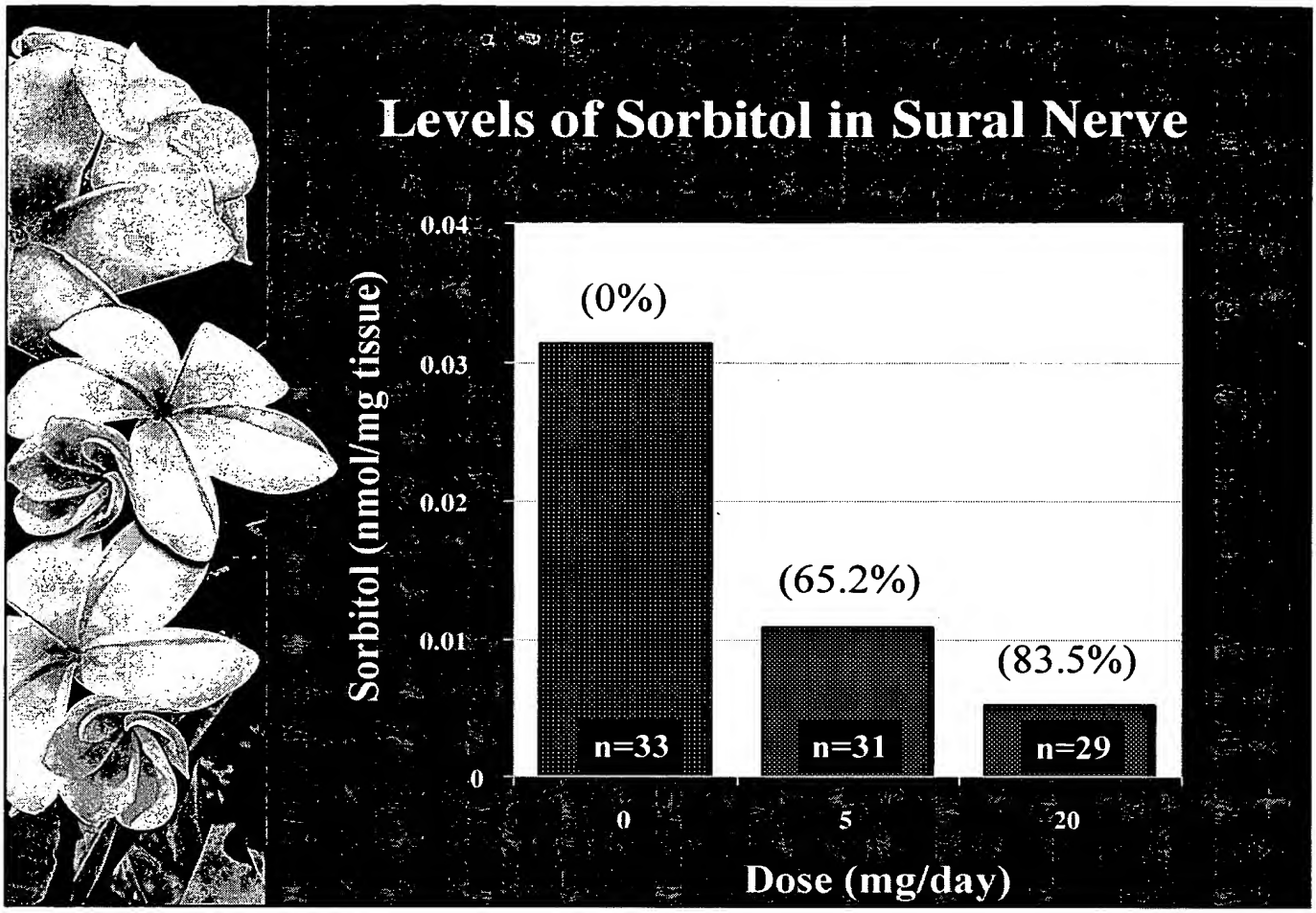
Plasma AS-3201 Concentrations (ng/mL)	Placebo	Treatment Group AS-3201 5 mg	20 mg
Baseline	ND	ND	ND
Week 2	ND	209.84 ± 55.87	661.41 ± 265.79
Week 4	ND	218.44 ± 71.25	671.86 ± 323.79
Final Visit	ND	213.94 ± 66.28	717.99 ± 372.12

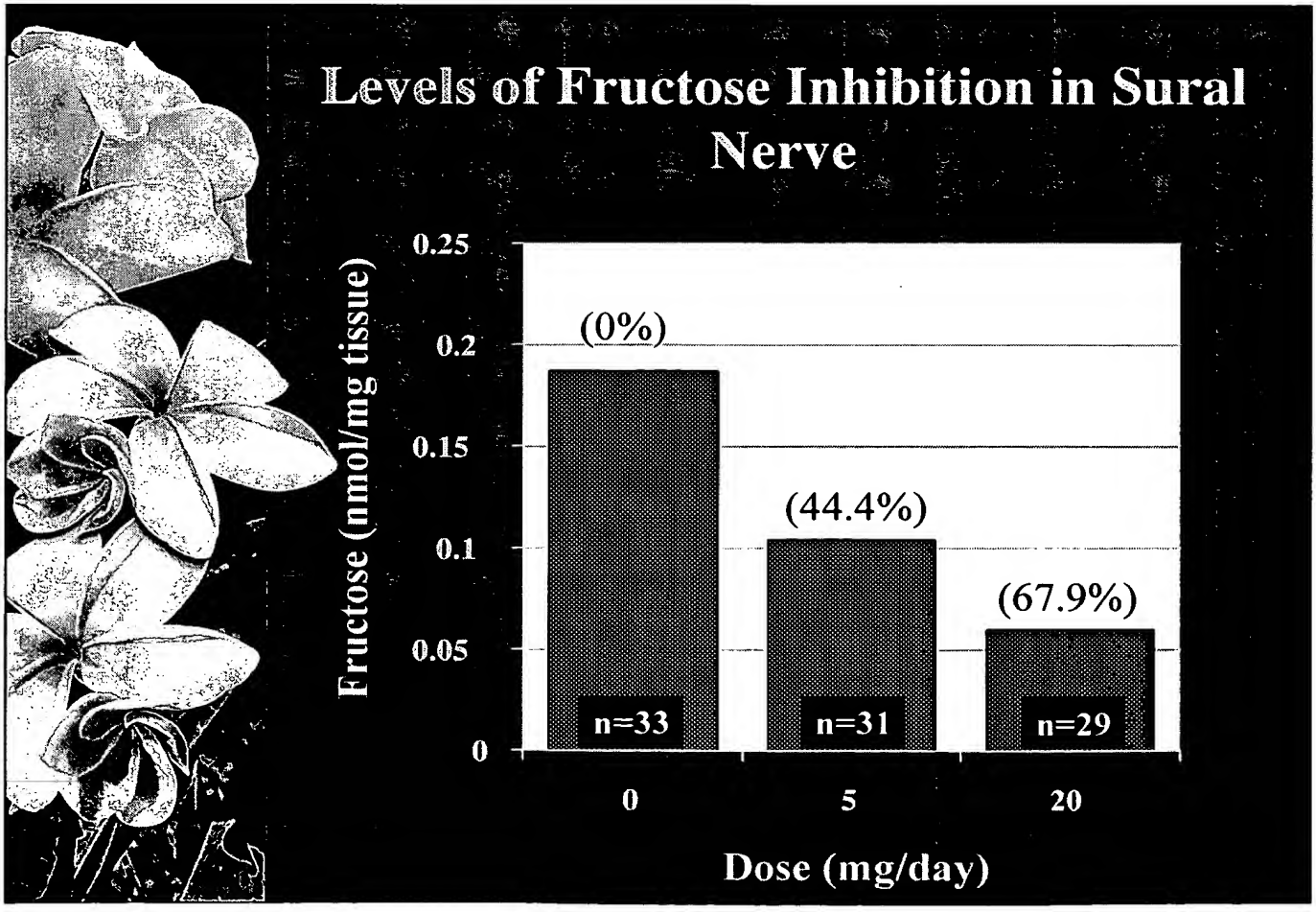
Values expressed as mean ± standard deviation
ND for not detectable (<2 ng/mL)

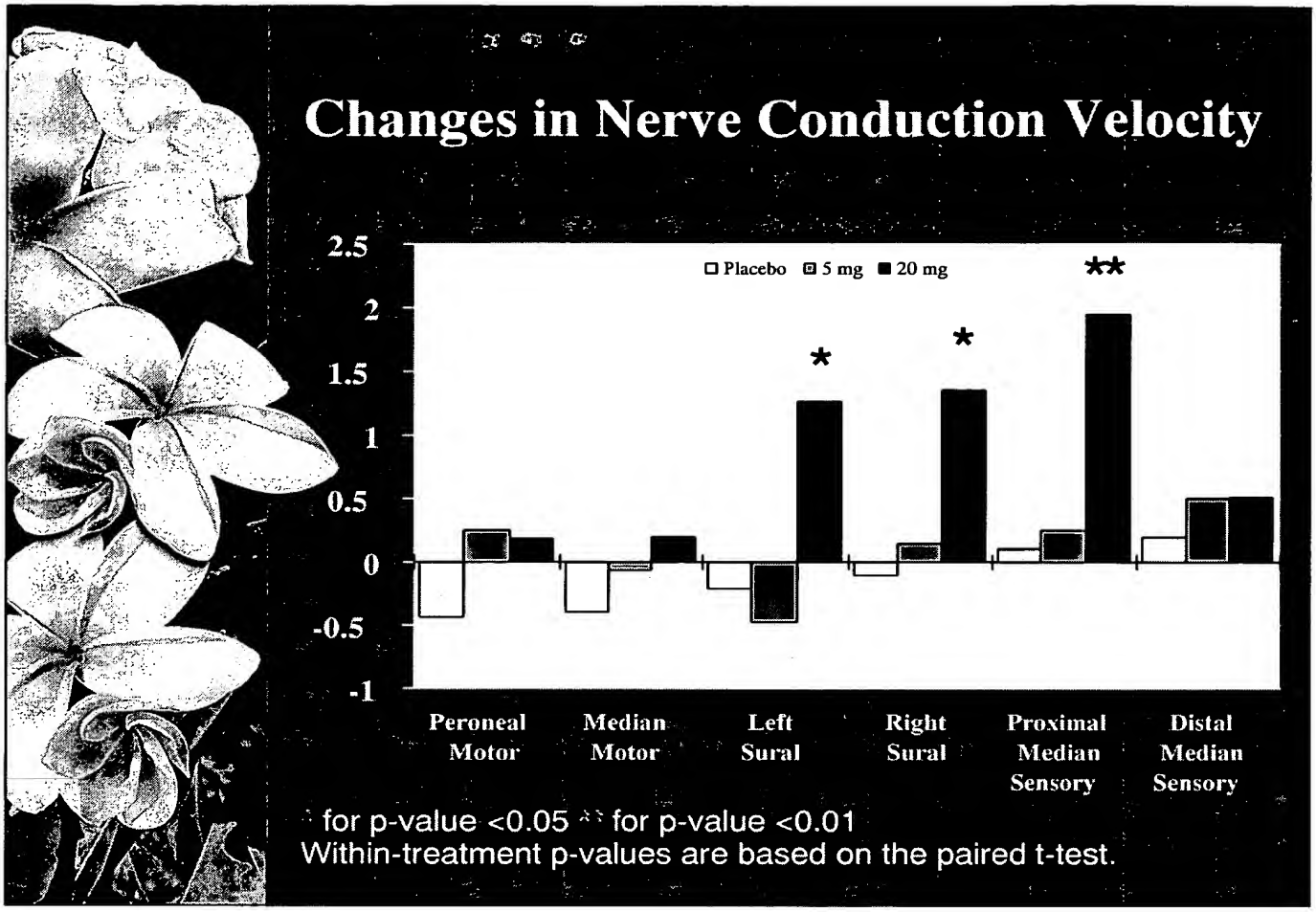
Sural Nerve Sorbitol, Fructose & Nerve AS-3201 Levels

	Placebo n = 33	Treatment Group AS-3201 5 mg n = 31	20 mg n = 29
Sorbitol Concentration (nmol x 10^{-2}/mg wet nerve)	3.14 ± 3.58	1.09 ± 0.65*	0.52 ± 0.21*†
Reduction Rate, %	NA	65.2	83.5
Fructose Concentration (nmol x 10^{-2}/mg wet nerve)	18.69 ± 13.66	10.38 ± 6.72*	6.00 ± 3.65*‡
Reduction Rate, %	NA	44.4	67.9
AS-301 Concentration (ng/g wet nerve)	NA	108.37 ± 44.022	258.09 ± 108.113

* different from placebo, $p < 0.001$ based on ANOVA
† different from 5 mg dose, $p < 0.001$ based on ANOVA
‡ different from 5 mg dose, $p < 0.003$ based on ANOVA
Concentration values expressed as mean ± standard deviation

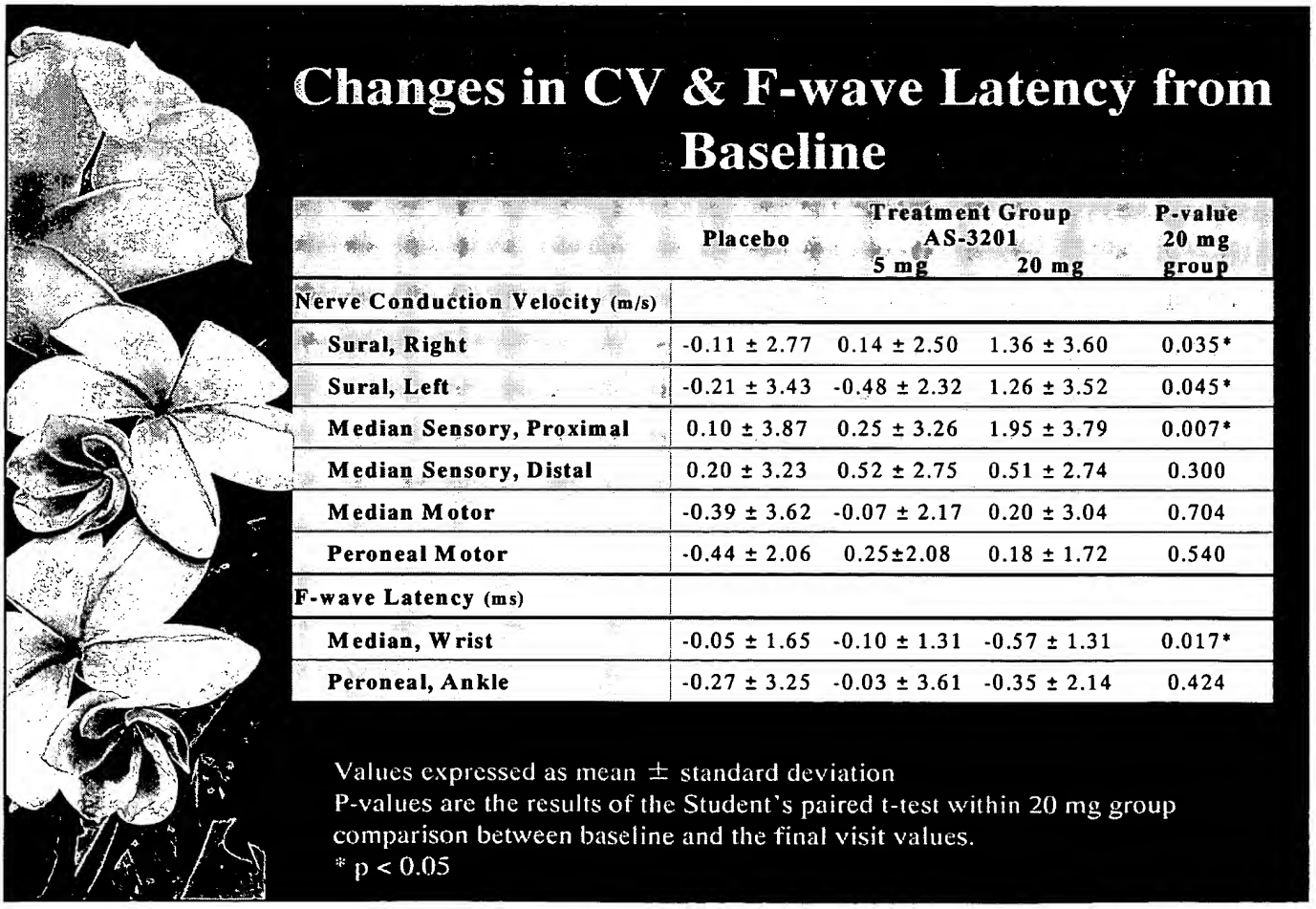

Levels of Sorbitol in Sural Nerve
Sorbitol (nmol/mg tissue)
0.04
0.03
0.02
0.01
0
(0%)
(65.2%)
(83.5%)
n=33
n=31
n=29
0
5
20
Dose (mg/day)


Levels of Fructose Inhibition in Sural Nerve
Fructose (nmol/mg tissue)
0.25
0.2
0.15
0.1
0.05
0
(0%)
(44.4%)
(67.9%)
n=33
n=31
n=29
0
5
20
Dose (mg/day)



Changes in Nerve Conduction Velocity

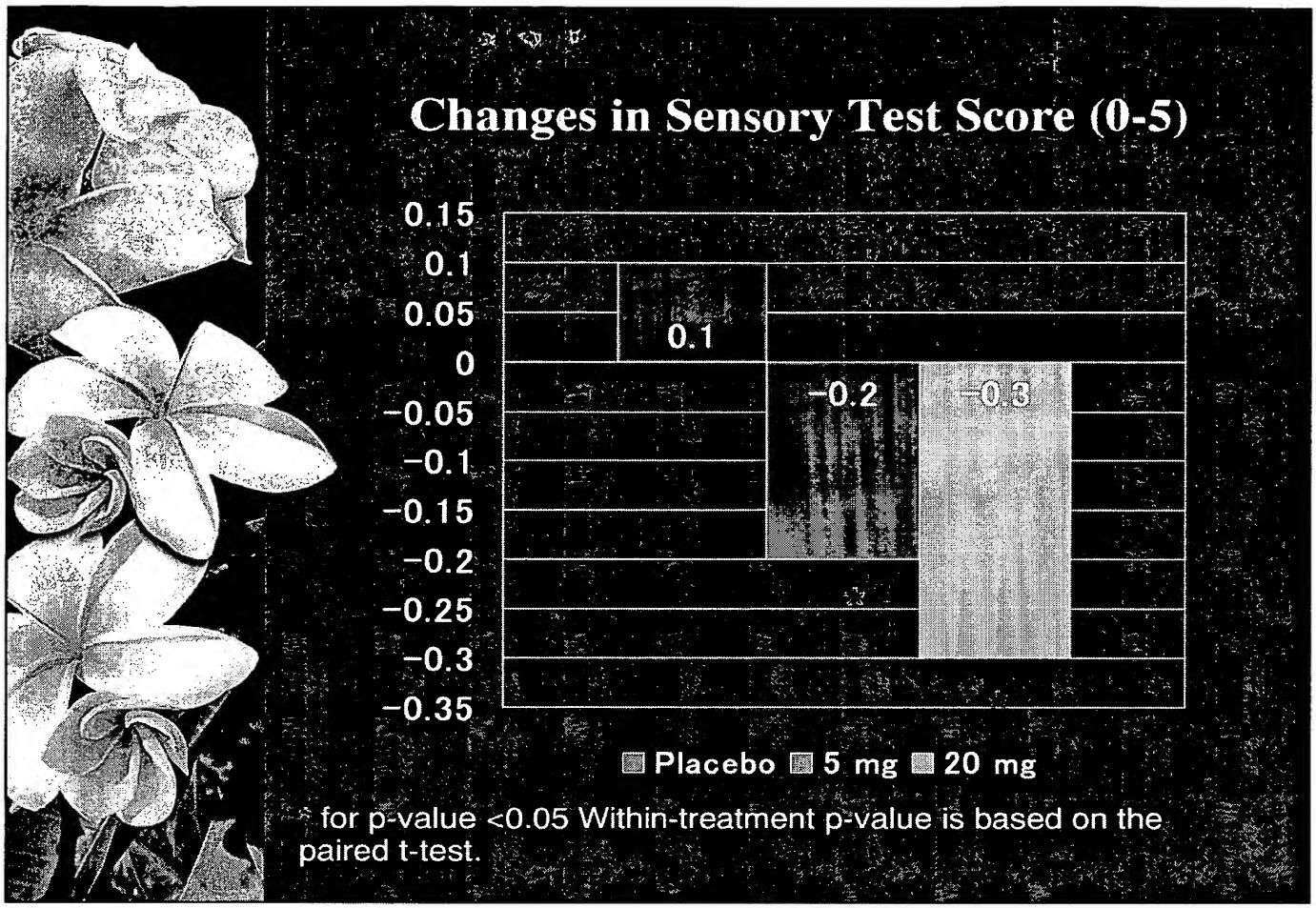
* for p-value <0.05 ** for p-value <0.01
Within-treatment p-values are based on the paired t-test.

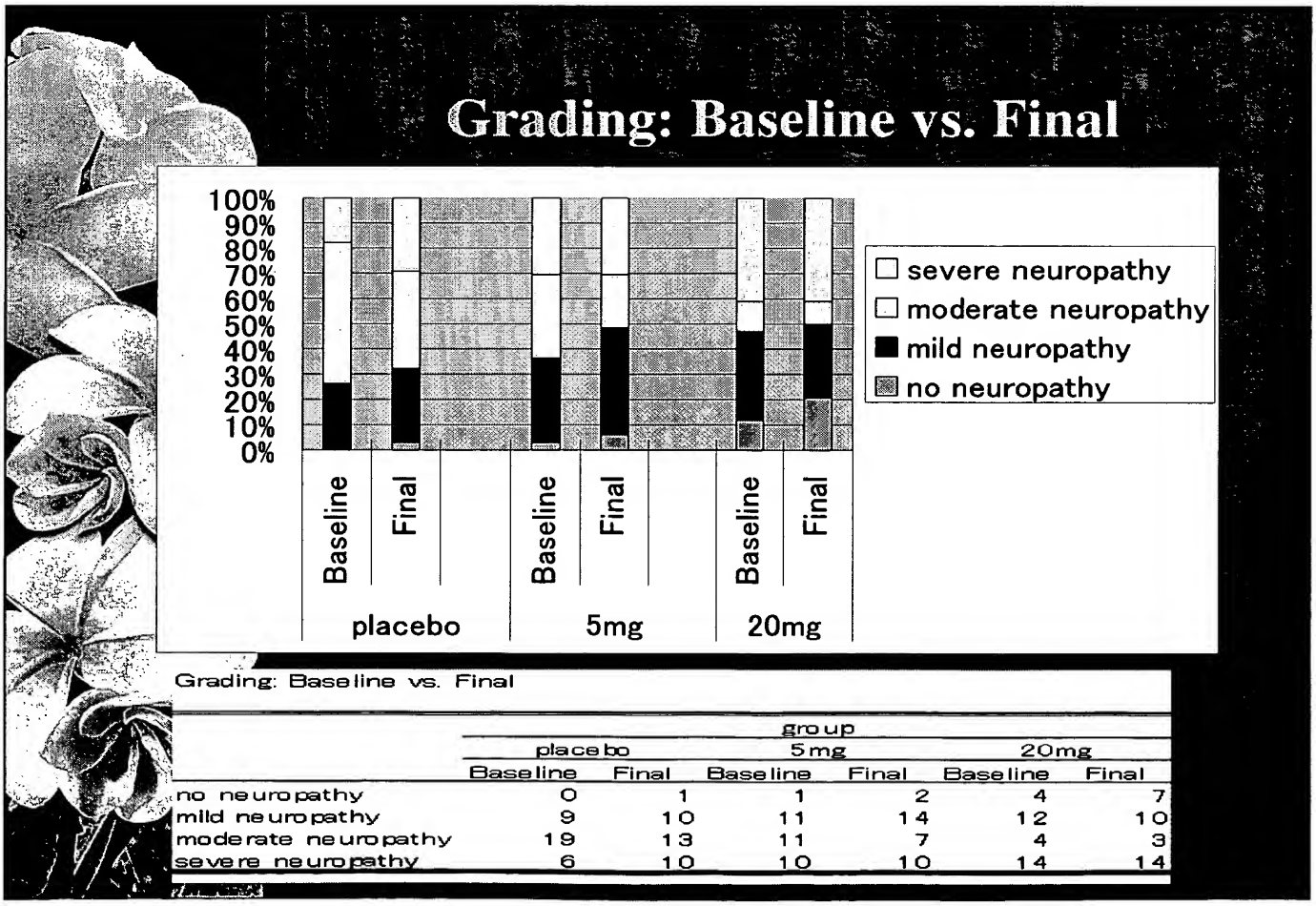


Changes in CV & F-wave Latency from Baseline

	Placebo	Treatment Group AS-3201 5 mg	Treatment Group AS-3201 20 mg	P-value 20 mg group
Nerve Conduction Velocity (m/s)				
Sural, Right	-0.11 ± 2.77	0.14 ± 2.50	1.36 ± 3.60	0.035*
Sural, Left	-0.21 ± 3.43	-0.48 ± 2.32	1.26 ± 3.52	0.045*
Median Sensory, Proximal	0.10 ± 3.87	0.25 ± 3.26	1.95 ± 3.79	0.007*
Median Sensory, Distal	0.20 ± 3.23	0.52 ± 2.75	0.51 ± 2.74	0.300
Median Motor	-0.39 ± 3.62	-0.07 ± 2.17	0.20 ± 3.04	0.704
Peroneal Motor	-0.44 ± 2.06	0.25±2.08	0.18 ± 1.72	0.540
F-wave Latency (ms)				
Median, Wrist	-0.05 ± 1.65	-0.10 ± 1.31	-0.57 ± 1.31	0.017*
Peroneal, Ankle	-0.27 ± 3.25	-0.03 ± 3.61	-0.35 ± 2.14	0.424

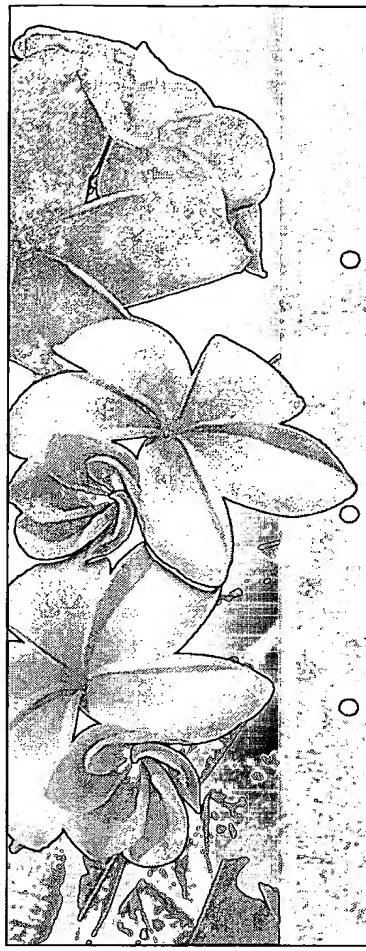
Values expressed as mean ± standard deviation
P-values are the results of the Student's paired t-test within 20 mg group comparison between baseline and the final visit values.
* $p < 0.05$





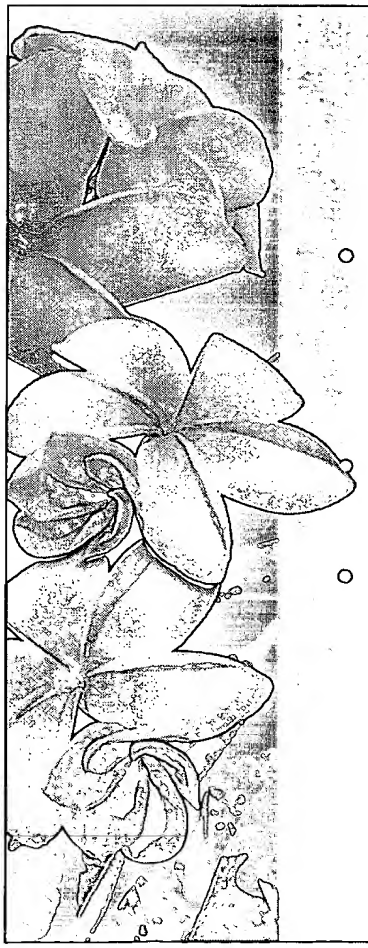
Grading: Baseline vs. Final

	group					
	placebo		5mg		20mg	
	Baseline	Final	Baseline	Final	Baseline	Final
no neuropathy	0	1	1	2	4	7
mild neuropathy	9	10	11	14	12	10
moderate neuropathy	19	13	11	7	4	3
severe neuropathy	6	10	10	10	14	14



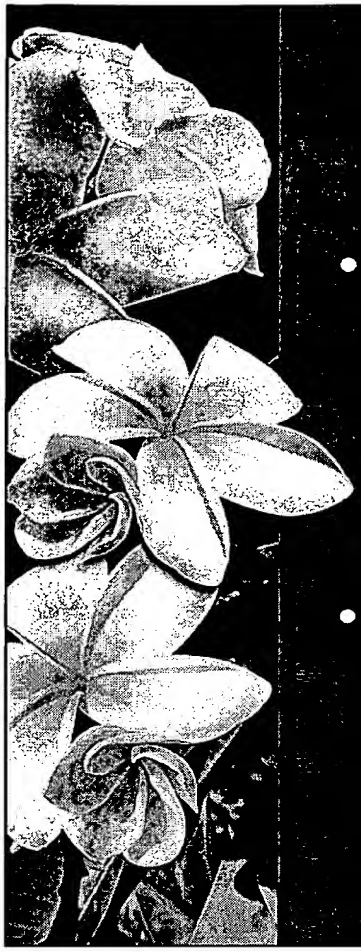
Summary

- The sural nerve sorbitol and fructose content were significantly lower in the active treatment groups versus the placebo group.
- The inhibition of polyol accumulation in the sural nerve with AS-3201 was dose-dependent.
- AS-3201 penetrated human sural nerve tissue 2.4 x more in the 20 mg/d group compared to the 5 mg/d group.



Summary

- The baseline to final changes in sensory nerve conduction velocities, and median F-wave latency attained statistical significance within the 20 mg/d AS-3201 group.
- Trends to improvement were observed in subsections of the TCNS
- The proportion of patients graded as no neuropathy increased in the 20 mg/d AS-3210 group.



Conclusions

- 12 weeks of treatment with AS-3201
  - inhibits the polyol pathway in sural nerve from patients with DSP
  - improves nerve function
  - tends to improve some clinical measures
- AS-3201 is a potent ARI